Hepatocyte growth factor, Met, and CD44. Amenage a trois in B cells
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Citation for published version (APA):
van der Voort, R. (2000). Hepatocyte growth factor, Met, and CD44. Amenage a trois in B cells
Chapter 1

General introduction
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Introduction

The immune system serves to protect organisms against pathogens such as bacteria, viruses, and parasites. Based on functional grounds, it can be divided in two branches: the innate and the adaptive immune system. The innate immune system is constitutional and acts as a first line of defence. It involves soluble factors, natural killer cells, and phagocytes, and does not improve on repeated contact with the same pathogenic factor (antigen [Ag]). The adaptive immune system, by contrast, will evoke a highly specific immune reaction. In addition, it will develop an immunological memory which will provide a quicker and enhanced immune response upon restimulation with the Ag. The most important players during an adaptive immune response are the T and B lymphocytes. While T lymphocytes are mainly involved in the destruction of infected cells, and in the regulation of the immune response, B cells are the producers of immunoglobulins (Igs). These Igs specifically recognize and bind Ag, leading to the destruction of the organisms or cells that express the Ag. Most B cells express Igs at their cell surface in a complex with several other proteins, the B cell antigen receptor (BCR). However, if B cells are activated by antigen, and differentiate into plasma cells, they no longer express BCRs, but secrete the Igs instead. Igs are composed of the unique combination of

![Image of Immunoglobulin](image_url)

**Figure 1.** Schematic representation of an immunoglobulin (Ig). Two Ig heavy (IgH) chains pair with two Ig light (IgL) chains to form two identical antigen-binding sites. The constant (C_H or C_L) and variable (V_H or V_L) regions, the complementarity determining regions (CDRs), the framework regions (FRs), the hinge region (H), the antigen binding site, and the sulphate bridges (SS) are shown.
two identical heavy (IgH) and two identical light chains (IgL) (Fig. 1). Both the IgH and IgL chains consist of constant (C\text{H} or C\text{L}) and variable (V\text{H} or V\text{L}) regions (Fig. 1). While C regions mediate the expression at the cell membrane, the binding of complement, and the interaction with Ig-receptors on other cells, V regions interact with the Ag. The recognition of Ag is mainly mediated by three hypervariable domains of the V regions, the complementarity determining regions (CDRs) (Fig. 1). These CDRs are separated by less variable framework regions (FRs) which maintain the stability of the V regions (Fig. 1).

**B cell development**

Primary B cell development takes place in the bone marrow. Here, (committed) lymphoid progenitors differentiate into immature B cells. An important process during early B cell development is the rearrangement of the variable (V), diversity, and joining (J) gene segments of the IgH gene locus (Tonegawa, 1983; Alt et al., 1987). This will allow the B cell to express the pre-BCR, which is composed of the rearranged IgH chain complexed to a surrogate IgL chain (Melchers et al., 1994). Successful expression of the pre-BCR is indispensable for survival of the B cell. B cells lacking a pre-BCR, or expressing an aberrant pre-BCR, will die by apoptosis (Melchers, 1999). Signaling via the pre-BCR will induce rearrangement of the V and J gene segment of the IgL gene locus (Ten Boekel et al., 1995; Constantinescu and Schlissel, 1997). Adequate rearrangement, and successful complexation with the IgH chain, are required for B cell survival, and allows the B cell to express a BCR of the IgM isotype (Melchers et al., 1994).

The IgM-expressing B cell will now leave the bone marrow, and travel via the blood to secondary lymphoid organs, i.e. the spleen, lymph nodes, and mucosal-associated lymphoid tissue (MALT). In these organs, the B cells may be confronted with native Ag that is recognized by their cell surface-expressed immunoglobulins which form the B cell antigen receptor (BCR). In case of recognition, the B cells will internalize and process the Ag. At the border of the T cell area and the B cell area (follicle), the B cells will now present Ag-derived peptides on major histocompatibility complex (MHC) class II molecules to antigen receptors (TCRs) expressed by T helper cells that have been activated by dendritic cells (Lindhout et al., 1997; MacLennan et al., 1997; Steinman et al., 1997; Banchereau and Steinman, 1998; Garside et al., 1998). Upon recognition of the right peptide/MHC combination, the T cell will provide the B cell with co-stimulatory signals which are indispensable for further differentiation. These signals have been shown to critically depend on the interaction between CD40 and CD40L, and between CD28 and CD86 (Foy et al., 1994; Gray et al., 1994; Renshaw et al., 1994; Facchetti et al., 1995; Han et al., 1995; Ferguson et al.,
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1996). The activated B cell can now develop into an antibody-producing plasma cell, or respond with a series of events known as the germinal center reaction (Fig. 2) (MacLennan, 1994, 1997; Nossal, 1994; Kelsoe et al., 1995; Kosco-Vilbois et al., 1997; Lindhout et al., 1997; Liu and Arpin, 1997). This process, which will lead to affinity maturation of the humoral immune response, is initiated by the migration of activated B cells into the primary follicle of the secondary lymphoid organ (Liu et al., 1991; Jacob et al., 1991a). Here, the activated B cells will initiate the development of a germinal center (GC). On histological grounds, GC are subdivided into a dark- and a light zone (Fig. 2) (Liu et al., 1992). In the GC dark zone, the B cells, which have become centroblasts, undergo rapid clonal expansion and start to somatically hypermutate the variable regions of their Ig genes (Berek et al., 1991; Jacob et al., 1991b; Küppers et al., 1993; McHeyzer-Williams et al., 1993; Pascual et al., 1994; Kelsoe, 1996). Camacho et al. (1998) demonstrated that in mice, clonal expansion and somatic hypermutation take place in the GC light zone as well. After expansion, and IgV hypermutation, the centroblasts migrate to the basal part of the germinal center light zone and differentiate into centrocytes. In the light zone the centrocytes will start to express their mutated slgs, and re-encounter their Ag in the form of immune complexes presented by follicular dendritic cells (FDCs) (Gray et al., 1984; Liu et al., 1992, 1996b; Caux et al., 1995; Lindhout et al., 1997; Liu and Arpin, 1997; Banchereau and Steinman, 1998). During this process, the apoptosis-sensitive centrocytes will become affinity selected (Liu et al., 1989; Tew et al., 1990; Hardie et al., 1993; Lindhout et al., 1997). Centrocytes expressing slgs with a high affinity for the Ag will survive, while centrocytes with low affinity slgs, and autoreactive centrocytes, will die by apoptosis. It has been demonstrated that strong adhesion, involving the adhesion molecules LFA-1 - ICAM-1, and α4β1 VCAM-1 (see section Adhesion molecules and their ligands), is required for an effective interaction between the centrocyte and the FDC in vitro (Koopman et al., 1991, 1994; Lindhout et al., 1993). After their rescue from apoptosis, the centrocytes will retrieve and process some of the Ag, and present Ag-derived peptides in the context of MHC class II proteins to Ag-specific T cells present in the apical light zone of the GC. If these T cells, which have previously been activated by dendritic cells (Grouard et al., 1996), recognize the peptide-loaded MHC, they will provide the B cells with co-stimulatory signals via CD40-CD40L interaction and cytokine production (Lederman et al., 1992; Fuller et al., 1993; Casamayor-Palleja et al., 1995; Zheng et al., 1996). In addition, CD40-CD40L binding will downregulate the expression of the apoptosis-inducing FasL protein on the T cells (Rathmell et al., 1996). The stimulatory signals will eventually lead to Ig isotype switching and differentiation of the centrocytes into either memory B cells or Ig-secreting plasma cells (Kraal et al., 1982; Arpin et al., 1995; Liu et al., 1996a; Malisang et al., 1996). Finally, the
fully differentiated Ag-specific B cells will receive signals that mediate their export from the lymphoid tissues.

Figure 2. Schematic representation of the consecutive developmental steps that take place during the T cell-dependent B cell differentiation in secondary lymphoid organs. See the text for details. B, B cell; FDC, follicular dendritic cell; T, T cell.

Adhesion and migration of B cells

Adhesion and migration are essential for the differentiation and functioning of B cells, since these cells have to migrate between different lymphoid tissues, and have to interact with many cell types. Due to the large number of possible migration pathways and cellular interactions, the adhesion and migration of B cells have to be tightly regulated. The cells of the immune system are equipped with a number of molecules that perform this task. While adhesion molecules and their ligands are involved in the adhesion and migration of B cells, several cytokines, in particular members of the chemokine family, attract B cells and in this way determine the direction of their migration. In the next sections these molecules will be discussed.
Adhesion molecules and their ligands

**Selectins**

The selectin family consists of three members: L-selectin, E-selectin and P-selectin (Table I) (for review see Lasky, 1992, 1995; Bevilacqua 1993; Rosen et al., 1994; McEver et al., 1995; Tedder et al., 1995; Rossiter et al., 1997). Structurally, they are characterized by the presence of an N-terminal lectin domain, an epidermal growth factor-like domain, a variable number of tandem consensus repeats homologues to complement-binding domains, and a transmembrane and short cytoplasmic domain (Fig. 3) (Bevilacqua et al., 1989; Johnston et al., 1989; Lasky et al., 1989; Siegelman et al., 1989; Tedder et al., 1989). Whereas E-selectin is expressed by cytokine-activated endothelial cells, P-selectin is present in the Weibel-Palade bodies of endothelial cells and in α-granules of platelets (Hsu-Lin et al., 1984; Stenberg et al., 1985; Pober et al., 1986; Bevilacqua et al., 1987; Bonfanti et al., 1989; Johnston et al., 1989; Leeuwenberg et al., 1989; McEver et al., 1989). Upon cytokine-activation P-selectin becomes rapidly, but transiently, expressed on the cell-surface (Hsu-Lin et al., 1984; McEver et al., 1989). In contrast to E- and P-selectin, L-selectin is expressed on lymphocytes and myeloid cells (Gallatin et al., 1983; Tedder et al., 1989, 1990; Bowen et al., 1990).

In the presence of calcium ions, selectins bind to sugar moieties present on glycoproteins (sialomucins) expressed on the surface of leukocytes or endothelial

![Figure 3. Schematic representation of the four major families of adhesion molecules (not drawn to scale) involved in leukocyte adhesion and migration. See the text for details.](image-url)
Table I. The selectin family of adhesion molecules.

<table>
<thead>
<tr>
<th>Name</th>
<th>CD</th>
<th>Expression</th>
<th>Ligand(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-selectin</td>
<td>CD62L</td>
<td>leukocytes</td>
<td>GlyCAM-1, CD34,</td>
<td>Lasky et al., 1992; Baumhueter et al., 1993; Berg et al., 1993; Guyer et al., 1996; Spertini et al., 1996; Tu et al., 1996; Sassetti et al., 1998</td>
</tr>
<tr>
<td>E-selectin</td>
<td>CD62E</td>
<td>activated endothelium</td>
<td>ESL-1, PSGL-1; CLA</td>
<td>Berg et al., 1991; Levinovitz et al., 1993; Walcheck et al., 1993; Alon et al., 1994; Asa et al., 1995; Steegmaier et al., 1995</td>
</tr>
<tr>
<td>P-selectin</td>
<td>CD62P</td>
<td>activated endothelium, platelets</td>
<td>PSGL-1</td>
<td>Moore et al., 1992; Sako et al., 1993; Alon et al., 1994</td>
</tr>
</tbody>
</table>

CD, cluster of differentiation; CLA, cutaneous lymphocyte-associated antigen; E-selectin, endothelial selectin; ESL, E-selectin ligand; GlyCAM, glycosylation-dependent cell adhesion molecule; L-selectin, leukocyte selectin; MAdCAM, mucosal addressin cell adhesion molecule; PCLP, podocalyxin-like protein; P-selectin, platelet selectin; PSGL, P-selectin glycoprotein ligand.

cells (Lasky, 1992, 1995; Nelson et al., 1995). Sialic acid, fucose, and sulfate have been demonstrated to be essential components of most of the carbohydrate structures recognized by the selectins (Brandley et al., 1990; Lowe et al., 1990; Walz et al., 1990). These structures have been shown to be closely related to the tetrasaccharides sialyl Lewis x (sLe\(^x\)) and sialyl Lewis a (sLe\(^a\)) (Lasky, 1995). The carbohydrate ligands for the selectins are linked to several mucin-like molecules. For instance, L-selectin binds to saccharides attached to CD34, or to saccharides of the secreted glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1), while all three selectins are able to interact with sugars on P-selectin glycoprotein ligand 1 (PSGL-1) (Table I) (Lasky et al., 1992; Moore et al., 1992; Baumhueter et al., 1993; Sako et al., 1993; Asa et al., 1995; Puri et al., 1995; Guyer et al., 1996; Spertini et al., 1996; Tu et al., 1996). Moreover, E-selectin interacts also with E-selectin ligand 1 (ESL-1), and with the cutaneous lymphocyte antigen (CLA) expressed by skin-homing T cells (Berg et al., 1991; Levinovitz et al., 1993; Walcheck et al., 1993; Asa et al., 1995; Steegmaier et al., 1995).

Selectins and their ligands are mainly involved in tethering (bouncing) and rolling of leukocytes on the endothelium (see also the section Lymphocyte
**Table II. Immunoglobulin superfamily members involved in leukocyte adhesion**

<table>
<thead>
<tr>
<th>Name</th>
<th>CD</th>
<th>Expression</th>
<th>Ligand(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1</td>
<td>CD5-1</td>
<td>leukocytes, endothelium, dendrite cells</td>
<td>αLβ2, αMβ2, CD13</td>
<td>Makgoba et al., 1988; Staunton et al., 1988; Diamond et al., 1990; Staunton et al., 1989</td>
</tr>
<tr>
<td>ICAM-2</td>
<td>CD102</td>
<td>leukocytes, endothelium</td>
<td>αLβ2</td>
<td>Fawcett et al., 1992; Vareux et al., 1992; Gentenbeek et al., 2000</td>
</tr>
<tr>
<td>ICAM-3</td>
<td>CD50</td>
<td>leukocytes</td>
<td>αLβ2, DC-SIGN</td>
<td></td>
</tr>
<tr>
<td>ICAM-4</td>
<td>nd</td>
<td>erythrocytes</td>
<td>αLβ2</td>
<td>Batly et al., 1995</td>
</tr>
<tr>
<td>ICAM-5</td>
<td>nd</td>
<td>telencephalic neurons</td>
<td>αLβ2</td>
<td>Tian et al., 1997; Morano et al., 1997</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>CD106</td>
<td>activated endothelium, dendrite cells</td>
<td>α4β1</td>
<td>Oshorn et al., 1989; Elias et al., 1990</td>
</tr>
<tr>
<td>MAdCAM-1</td>
<td>nd</td>
<td>mucosal endothelium, mucosal dendritic cells</td>
<td>α4β7, L-selectin</td>
<td>Briskin et al., 1993; Berg et al., 1993; Berlin et al., 1993; Shyan et al., 1996; Szabo et al., 1997</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>CD31</td>
<td>leukocytes, endothelium</td>
<td>PECAM-1, αβ3, GAGs</td>
<td>Albida et al., 1991; Maller et al., 1992; DeLisser et al., 1993</td>
</tr>
<tr>
<td>ALCAM</td>
<td>CD166</td>
<td>activated T cells, B cells, monocytes</td>
<td>ALCAM, CD6</td>
<td>Uehida et al., 1997; Bowen et al., 1995</td>
</tr>
</tbody>
</table>

AlCAM, activated leukocyte cell adhesion molecule; CD, cluster of differentiation; GAG, glycosaminoglycan; ICAM, intercellular cell adhesion molecule; LFA, lymphocyte function-associated antigen; L-selectin, leukocyte selectin; MAdCAM, mucosal addressin cell adhesion molecule; nd, not determined; PECAM, platelet-endothelial cell adhesion molecule; VCAM, vascular cell adhesion molecule.

**Extravasation: a multistep process.** Several characteristics make these molecules well suited for these loose and reversible interactions. For example, the bonds between selectins and their carbohydrate ligands have low association and dissociation constants. Moreover, the high concentration of L-selectin and other tethering receptors, e.g. PSGL-1, on the tips of the lymphocyte's microvilli enhances the efficiency of the brief interactions with their ligands (von Andrian et al., 1995). Finally, the rapid cleavage of L-selectin from the cell-surface upon
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cross-linking, ensures the reversibility of the binding process (Palecanda et al., 1992).

**Immunoglobulin superfamily**
The members of the immunoglobulin superfamily (IgSF) are characterized by the presence of so-called immunoglobulin (Ig) domains. These Ig-domains are composed of about 100 amino acids folded in a loop by disulfide bonds (Fig. 3). Beside well-known family members like the IgGs themselves, the T cell receptor, CD3, and the major histocompatibility complexes, this family also contains several proteins involved in cell adhesion (Table II). IgSF adhesion molecules can be expressed by a broad array of cell types (Table II) (for review see Wang and Springer, 1998). For instance, intercellular cell adhesion molecule 1 (ICAM-1) can be expressed by leukocytes as well as by activated endothelial cells, dendritic cells, and epithelial cells (Dustin et al., 1986). Often the level of expression is regulated by inflammatory mediators, e.g. both IL-1 and interferon γ induce the the expression of ICAM-1 on endothelial cells (Dustin et al., 1986).

Many of the IgSF adhesion molecules are involved in the interaction between leukocytes and endothelial cells. During most of these interactions the IgSF members bind to activated integrins (Table II). Since these interactions are often of high affinity, they are mainly involved in the tight adhesion of leukocytes to the endothelium (see also the section *Lymphocyte extravasation: a multistep process*). Mucosal addressin cell adhesion molecule 1 (MAdCAM-1) is an expectional member in this respect, because this molecule binds both L-selectin via carbohydrate moieties, and the integrin α4β7 via Ig-domains (Berg et al., 1993; Berlin et al., 1993).

**Integrins**
The integrin family is composed of related type I transmembrane proteins (for review see Hynes, 1992; Schwartz et al., 1995; Shimizu et al., 1999). These proteins are heterodimers consisting of an α chain non-covalently associated with a β chain (Fig. 3). So far 17 α and 8 β subunits have been characterised in humans (Shimizu et al., 1999). Since most, if not all, β subunits can associate with several different partner subunits, more than 20 different heterodimers have been detected at present. Based on their common β chains, eight integrin subfamilies have been defined. Some of these subfamilies, e.g. the β1 subfamily, are widely expressed. Members of the β2 subfamily are only expressed by leukocytes (Table III). B cells are able to express the integrins α4β1, α5β1, αLβ2, and α4β7 (Freedman et al., 1990; Koopman et al., 1991; Roldan et al., 1992; Arroyo et al., 1996; Drillenburg
### Table III. The major integrins involved in leukocyte adhesion

<table>
<thead>
<tr>
<th>Name</th>
<th>Synonym(s)</th>
<th>CD</th>
<th>Expression</th>
<th>Ligand(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1β1</td>
<td>VLA-1</td>
<td>CD49α/CD29</td>
<td>broad</td>
<td>laminin, collagen</td>
<td>Goodman et al., 1991</td>
</tr>
<tr>
<td>α2β1</td>
<td>VLA-2</td>
<td>CD49b/CD29</td>
<td>broad</td>
<td>laminin, collagen</td>
<td>Elckes et al., 1989</td>
</tr>
<tr>
<td>α3β1</td>
<td>VLA-3</td>
<td>CD49c/CD29</td>
<td>broad</td>
<td>laminin, collagen, fibronecetin, epithrin</td>
<td>Carter et al., 1991; Lakada et al., 1991</td>
</tr>
<tr>
<td>α4β1</td>
<td>VLA-4</td>
<td>CD49d/CD29</td>
<td>broad</td>
<td>fibronecetin, VCAM-1</td>
<td>Elckes et al., 1990</td>
</tr>
<tr>
<td>α5β1</td>
<td>VLA-5</td>
<td>CD49e/CD29</td>
<td>broad</td>
<td>fibronecetin</td>
<td>Pytel et al., 1985</td>
</tr>
<tr>
<td>α6β1</td>
<td>VLA-6</td>
<td>CD49f/CD29</td>
<td>broad</td>
<td>laminin</td>
<td>Sonnenberg et al., 1988</td>
</tr>
<tr>
<td>αβ2</td>
<td>LFA-1</td>
<td>CD11a/CD18</td>
<td>leukocytes</td>
<td>ICAM-1, 2, 3, 4-6</td>
<td>Martin et al., 1987; Makgoba et al., 1988; Stainton et al., 1989; Laweit et al., 1992; Bailly et al., 1995; Tian et al., 1997</td>
</tr>
<tr>
<td>αβ2</td>
<td>Mac 1, CR3</td>
<td>CD11b/CD18</td>
<td>monocytes, macrophages, granulocytes, lymphocytes</td>
<td>ICAM-1, 2, fibrinogen, iC3b, Factor X, glycoprotein, lba, CD23</td>
<td>Alten and Edgington, 1988; Wirtz et al., 1988; Diamond et al., 1990; Leconnet-Henchor et al., 1995; Xie et al., 1995; Simon et al., 2000</td>
</tr>
<tr>
<td>αβ2</td>
<td>αβ2</td>
<td>CD11c/CD18</td>
<td>macrophages, monocytes, granulocytes</td>
<td>fibrinogen, iC3b, CD23</td>
<td>Macklem et al., 1985; Myones et al., 1988; Loike et al., 1991; Postigo et al., 1991; Leconnet-Henchor et al., 1995</td>
</tr>
<tr>
<td>αβ7</td>
<td>LPAM-1</td>
<td>CD49d/nd</td>
<td>lymphocytes</td>
<td>MAdCAM-1, fibronecetin, VCAM-1</td>
<td>Ruegg et al., 1992; Berlim et al., 1993; 1995</td>
</tr>
<tr>
<td>αβ7</td>
<td>HML-1</td>
<td>CD103/nd</td>
<td>lymphocytes</td>
<td>E cadherin</td>
<td>Cepek et al., 1994</td>
</tr>
</tbody>
</table>

1 CD, cluster of differentiation; E cadherin, epithelial cadherin; HML, human mucosal lymphocyte; ICAM, intercellular cell adhesion molecule; LFA, lymphocyte function-associated antigen; LPAM, lymphocyte Peyer's patch adhesion molecule; Mac, macrophage; MAdCAM, mucosal addressin cell
adhesion molecule: nd, not determined; VCAM, vascular cell adhesion molecule; VLA, very late activation antigen.

et al., 1997). However, their expression is dependent on the differentiation state and site of origin of the B cell (Horst et al., 1991; Shimizu et al., 1999; Butcher and Picker, 1996; Butcher et al., 1999).

Most integrins can bind to several different ligands (Table III). Many of these ligands are molecules of the extracellular matrix, e.g. fibronectin and laminin, while others are cell surface-expressed members of the immunoglobulin superfamily, e.g. ICAM-1 and VCAM-1 (see Immunoglobulin superfamily). In order to bind their ligand, integrins require metabolic energy, the presence of extracellular Ca\(^{2+}\) and/or other divalent cations like Mg\(^{2+}\) or Mn\(^{2+}\) (Hynes, 1992; Schwartz et al., 1995; Shimizu et al., 1999). Generally, leukocyte integrins reside in a resting state and have to be activated before they can bind their ligand. This activation may involve enhanced affinity due to a conformational change of the integrin, and/or enhanced avidity resulting from the recruitment of integrin molecules in adhesion complexes (Shimizu et al., 1999). Many studies have indicated that activation of signaling molecules, and alterations in the organization of the cytoskeleton are involved in integrin-dependent adhesion (Schwartz et al., 1995; Hemler, 1998; Giancotti and Ruoslahti, 1999). The mechanisms involved in these (transient) processes are as yet not completely clear. However, it has been demonstrated that activation of several cell surface receptors, including the TCR, MHC class II, and CD44, leads to activation of integrins (Dustin and Springer, 1989; van Kooyk et al., 1989; Koopman et al., 1990; Mourad et al., 1990; Alexander et al., 1993; Vermotdesroches et al., 1995). In addition, cytokines, in particular the chemokines, have been shown to induce integrin-dependent adhesion (Schwartz et al., 1995; Baggiolini, 1997, 1998) (see also the section Regulation of B cell adhesion and migration). Hence, intracellular signals evoked by cell surface-expressed receptors can lead to integrin activation, a process known as inside-out signaling (Hynes, 1992; Schwartz et al., 1995). Alternatively, outside-in signaling, i.e. stimulation of integrins via external factors, can lead to signals that influence many biological processes like adhesion, proliferation, cytokine production, and apoptosis (Hynes, 1992; Koopman et al., 1992, 1994; Schwartz et al., 1995; van Kooyk et al., 1998; Shimizu et al., 1999).

**CD44**

CD44 represents a large family of cell-surface expressed glycoproteins encoded by one gene which consists of 19 exons (Screaton et al., 1992; Lesley et al., 1993) (Figs. 3 and 4). Through alternative RNA-splicing, involving at least 10 exons,
many different CD44 isoforms can be generated (Goldstein et al., 1989; Dougherty et al., 1991; Günthert et al., 1991; Stamenkovic et al., 1989, 1991; Jackson et al., 1992; Tölg et al., 1993). In addition to alternative splicing, post-translational modifications contribute to the diversity of the CD44 family. These modifications can result in isoforms modified with N- and/or O-linked glycans, and/or glucosaminoglycans (GAG) chains like chondroitin sulfate, heparan sulfate and dermatan sulfate (Jalkanen et al., 1988; Brown et al., 1991; Jackson et al., 1995; Takahashi et al., 1996). Members of the CD44 family can be expressed by almost all cell types (Lesley et al., 1993). Most cells express the 90 kDa "standard" (CD44s) CD44 isoform. This protein is the smallest CD44 isoform and does not contain any of the alternative domains. However, several cell types, including activated lymphocytes and endothelial cells, epithelial cells, and tumor cells, also express CD44 splice variants with molecular weights of up to 200 kDa (Pals et al., 1989b; Dougherty et al., 1991; Günthert et al., 1991; Hofmann et al., 1991; Stamenkovic et al., 1991; Arch et al., 1992; Jackson et al., 1992; Heider et al., 1993; Koopman et al., 1993; Fox et al., 1994; Mackay et al., 1994; Griffioen et al., 1997).

Through alternative splicing of exon 18, CD44 proteins can have a short (3 amino acids) or long (70 amino acids) cytoplasmic tail (Fig. 4) (Goldstein et al., 1989; Stamenkovic et al., 1989; Sereaton et al., 1992). CD44 isoforms with the long cytoplasmic tail have been shown to interact with several components of the cytoskeleton, including actin, ankyrin, and the linker proteins ezrin, radixin, and moesin (Tarone et al., 1984; Lacy and Underhill, 1987; Kalomiris and Bourguignon, 1988; Isacke, 1994; Tsukita et al., 1994). The association between CD44 proteins and the cytoskeleton has been suggested to regulate the binding between CD44 and its extracellular ligand hyaluronic acid (HA, see below) (Lesley et al., 1992; Thomas et al., 1992). In addition, cross-linking of CD44, or binding to HA, have been demonstrated to induce signal transduction resulting in, for instance, proliferation, adhesion or cytokine production (Hu et al., 1989; Shimizu et al., 1989; Koopman et al., 1990; Rothman et al., 1991). As yet, not much is known about the signal transduction pathways activated by CD44. However, in T cells CD44-induced signal transduction was shown to involve the Src family tyrosine kinases lyn and fyn (Täher et al., 1996; Ilangumaran et al., 1998). Recently, Ras, PKC, and IκB were demonstrated to be involved in the activation of NF-κB induced by the binding of CD44 to HA (Fitzgerald et al., 2000).

The CD44 family has been implicated in a number of important physiological and pathological processes (Lesley et al., 1993). For instance, CD44 isoforms play a role in migration, lymphocyte activation, hematopoiesis, development, and autoimmune diseases (Jalkanen et al., 1986, 1987; Koopman et al., 1990; Miyake et al., 1990a; Haynes et al., 1991; Camp et al., 1993; Wheatley
Figure 4. Schematic representation of the CD44 gene and proteins it encodes. As a result of alternative mRNA splicing both the extracellular domain and the cytoplasmic tail can vary in size. The alternatively spliced exons are indicated by open boxes. The human v1 exon contains a stop codon. All (putative) glycosylation sites of the CD44 protein are indicated: O-linked glycosylation sites (open circles); N-linked glycosylation sites (closed circles); chondroitin sulphate-attachment sites (open diamonds); heparan sulphate (HS)-attachment site (rod). In addition, the hyaluronic acid-binding sites (black lines), the disulfide bonds (S-S), the ankyrin-binding site (---), the ezrin-binding site (gray line), the phosphorylation sites (P), and the putative interaction site for the src-family kinase p56\(^{\text{lck}}\) are indicated.
et al., 1993; Estess et al., 1998; Sherman et al., 1998; Brocke et al., 1999; Siegelman et al., 1999; Weiss et al., 2000). Moreover, experimental and clinical data suggest that several CD44 isoforms are involved in tumorigenesis and metastasis (Herrlich et al., 1993; Naor et al., 1997; Drillenburg and Pals, 2000; Wielenga et al., 2000). For instance, CD44s was shown to enhance the migration of melanoma cells in vitro, as well as the local tumor formation and metastatic capacity of lymphoma cells in nude mice (Sy et al., 1991, 1992; Thomas et al., 1992). Interaction of CD44s with HA (see below) was shown to be important in these tumor models (Thomas et al., 1992; Bartolazzi et al., 1994). In other studies, expression of CD44 splice variants containing the domain encoded by exon v6 was shown to confer a metastatic potential to rat pancreatic carcinoma cells (Günther et al., 1991). Several clinical studies demonstrated that enhanced expression of CD44 by non-Hodgkin’s lymphomas is associated with tumor dissemination and an unfavorable prognosis (Pals et al., 1989a; Horst et al., 1990; Jalkanen et al., 1991; Stauder et al., 1995; Drillenburg and Pals, 2000). Furthermore, high expression of CD44 proteins by colorectal carcinomas was reported to be correlated with a more malignant tumor phenotype and with tumor-related death (Wielenga et al., 1993, 1998; Mulder et al., 1994, 1995; Orzechowski et al., 1995; Imazeki et al., 1996; Yamaguchi et al., 1996).

One extensively studied interaction is that between CD44, mainly CD44s, and the proteoglycan hyaluronic acid (HA) (Aruffo et al., 1990; Culty et al., 1990; Miyake et al., 1990b; Lesley et al., 1990, 1993; Kincade et al., 1997; Siegelman et al., 1999). HA is a major component of the extracellular matrix (ECM) and plays a role in cell migration and differentiation (Knudson and Knudson, 1993). The N-terminal CD44 domain, which is homologues to the HA-binding domains of the cartilage link and core proteins, mediates the binding to HA (Peach et al., 1993). Although some cell lines and tumor cells bind HA constitutively, CD44 on most normal cells needs to be activated before it can mediate adhesion to HA (Lesley et al., 1990, 1994; Murakami et al., 1990; Lesley and Hyman, 1992; Liao et al., 1993; DeGrendele et al., 1997b; Maita et al., 1998). So far, three distinct mechanisms have been shown to regulate CD44 – HA interaction: First, differences in glycosylation of CD44 can result in cell-type-dependent differences in HA binding. For instance, the presence of GAG chains, or N- or O-linked saccharides on CD44 proteins regulates their ability to bind HA (Katoh et al., 1995; Bartolazzi et al., 1996; Takahashi et al., 1996; English et al., 1998; Skelton et al., 1998). Also, sialylation of saccharides attached to CD44 was shown to be an important factor (Katoh et al., 1999). Furthermore, the proinflammatory cytokine tumor necrosis factor α (TNF-α) was demonstrated to convert CD44 from its inactive, nonbinding form to its active form by inducing sulfation of CD44, resulting in enhanced binding to HA and to vascular endothelial cells (Maiti et al., 1998). Second, alternative splicing of CD44 mRNA was shown to influence the affinity for HA.
presumably by inducing conformational changes or by attachment of saccharides to
*de novo* expressed CD-44 domains (Stamenkovic *et al*., 1991; Bennett *et al*., 1995a; this thesis). Third, clustering of CD-44 was shown to increase HA binding capacity (Seeman *et al*., 1996). Although some reports suggest that sequences in the cytoplasmic tail and/or transmembrane domain of CD-44 are also required for the binding to HA, this issue remains controversial (He *et al*., 1992; Lesley *et al*., 1992, 2000; Thomas *et al*., 1992; Isacke, 1994; Lokeshwar *et al*., 1994; Perschl *et al*., 1995; Püre *et al*., 1995; Uff *et al*., 1995; Liu and Sy, 1996; Leg and Isacke, 1998; Liu *et al*., 1998).

Functional studies indicate that triggering of the T cell receptor induces CD-44-dependent rolling of T cells on purified or endothelial-expressed HA (DeGrendele *et al*., 1996, 1997b; Siegelman *et al*., 1999) (see also the section *Lymphocyte extravasation: a multistep process*). In animal models this interaction was shown to play an important role in the extravasation of activated T cells into sites of inflammation (DeGrendele *et al*., 1997a).

In addition to HA, fibronectin, collagen, laminin, the cytokine osteopontin, and the proteoglycan serglycin were also reported to bind to CD-44 (Wayner and Carter, 1987; Jalkanen and Jalkanen, 1992; Faassen *et al*., 1992, 1993; Toyama-Sorimachi *et al*., 1995; Weber *et al*., 1996; Smith *et al*., 1999). As yet it is not known which specific CD-44 isoforms bind these molecules and what the functional consequence of their interaction is.

An interesting interaction is that between CD-44 and "heparin-binding" cytokines. Tanaka *et al*. (1993b) demonstrated that purified CD-44 molecules were able to bind the chemotactic cytokine (chemokine) macrophage inflammatory protein 3α (MIP-3α) and to present it to T cells. Presentation of MIP-3α resulted in enhanced integrin-dependent adhesion of the T cells and was shown to be HS-dependent. Also fibroblast growth factor 2 (FGF-2) and heparin-binding epidermal growth factor (HB-EGF) were reported to bind to CD-44 proteins in a heparan sulfate (HS)-dependent way (Bennett *et al*., 1995b). Moreover, binding and presentation of FGF-8 by HS-modified CD-44 (CD-44-HS) may be involved in limb bud formation during vertebrate development (Sherman *et al*., 1998). It was shown that only CD-44 isoforms expressing the domain encoded by exon v3 can be modified with HS and hence are able to bind cytokines like FGF-2 and HB-EGF (Bennett *et al*., 1995b; Jackson *et al*., 1995; this thesis). Covalent attachment of HS occurs at an evolutionary conserved SGSG consensus motif present in the CD-44v3 domain (Bourdon *et al*., 1987; Greenfield *et al*., 1999). The presence of several specific amino acids flanking the SGSG motif has been shown to be essential for the modification with HS (Greenfield *et al*., 1999). In addition to the attachment of HS, all CD-44 isoforms can be modified with the GAG chondroitin sulfate (CS) (Jalkanen *et al*., 1988). Modification with CS takes place at the SGSG site in the v3 domain, but also at an SG site present in the domain encoded by the constant CD-44
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Exon e5 (Greenfield et al., 1999). Interestingly, CS attached to CD44 mediates the binding of IFN-γ and probably some of its biological functions, including the suppression of cell proliferation, and the induction of major histocompatibility complex II antigens on vascular smooth muscle cells. The chemokine RANTES (regulated upon activation, normally T cell expressed and secreted) was shown to bind both CS and HS attached to recombinant CD44 molecules, resulting in enhanced T cell activation (Wolff et al., 1999).

Surprisingly, despite the broad expression and functionality of the CD44 family, Schmits and colleagues (1997) observed only minor defects in mice carrying a disrupted CD44 gene. Although these mice could not express any CD44 isoforms, they were born in a Mendelian ratio and showed no obvious developmental abnormalities. More detailed analysis demonstrated minor impairments in hematopoietic progenitor distribution and an exaggerated granuloma formation during infection. An independent study using CD44-deficient mice showed that two small T cell subsets, CD4+CD25+ and CD8+CD25+ cells, are lost in the thymus (Protin et al., 1999). Therefore, the authors hypothesized that CD44 isoforms are involved in the recirculation and/or survival of these T cell subsets. In addition, a study using mice bearing a keratinocyte-specific antisense CD44 transgene demonstrated that these mice suffered from a disrupted HA metabolism in their skin and an impaired proliferation of their keratinocytes (Kaya et al., 1997). These latter results indicate that for investigating the functions of the CD44 family, the generation of conditional knock-out mice is desirable.

Lymphocyte extravasation: a multistep process

Lymphocytes entering lymphoid tissues via the blood have to migrate between the endothelial cells lining the vessel wall, a process that generally takes place at specialized sites of the blood circulation known as high endothelial venules (HEVs). HEVs are located within secondary lymphoid organs, but often also develop at sites of inflammation (Pals et al., 1989c; Kraal and Mebius, 1997). HEVs are lined by cuboidal endothelial cells which are well equipped with adhesion molecules to facilitate lymphocyte transmigration. As shown in Fig. 5, lymphocyte extravasation is a multistep process (Butcher, 1991; Springer, 1994). During the first step leukocytes tether and subsequently roll on the endothelial cells of post-capillary venules. These events are transient and reversible, and mainly involve the (weak) interaction between selectins and their cognate carbohydrate ligands (sialomucins). Rolling slows the leukocyte down, and allows it to sample the environment for activating and/or chemotactic factors. If these factors, predominantly chemokines, are indeed present on the endothelial cells, this leads to
Figure 5. The multistep model of leukocyte extravasation. In postcapillary venules, the interaction between selectins and sialomucins mediates the rolling of leukocytes on the endothelium. Next, chemokines, probably presented by heparan sulphate proteoglycans, bind to their receptors expressed by the leukocytes. This leads to activation of integrins on the surface of the leukocyte. The integrins can now bind to members of the Ig superfamily expressed by the endothelial cells, resulting in firm adhesion, spreading, and transmigration.

the activation of the leukocyte, the second step. There is now increasing evidence that the activating chemokines are bound and presented by heparan sulfate proteoglycans (HSPGs) (see below) expressed by the endothelial cells. Chemokine presentation would highly increase the number of chemokine – receptor interactions, and consequently increase the chance to activate the leukocyte (Tanaka et al., 1993a). Activation of the leukocyte results in step three: strong and sustained adhesion, predominantly caused by the activation of integrins which now bind to members of the immunoglobulin superfamily (IgSF) expressed by the endothelial cells. The last step involves the transendothelial migration of the leukocyte. It has been suggested that the IgSF-member PECAM-1/CD31 (see section Adhesion molecules and their ligands – Immunoglobulin superfamily) plays an important role during this process (Muller et al., 1993; Wakelin et al., 1996).
Interestingly, the extravasation of lymphocytes is often tissue-specific, a phenomenon known as ‘homing’ (Springer, 1994; Butcher and Picker, 1996; Salmi and Jalkanen, 1997; Butcher et al., 1999). Homing of lymphocytes is mediated by adhesion molecules, the “vascular addressins”, expressed on endothelial cells present in specific regions of the body. So far, specific homing to the skin, gastrointestinal tract, central nervous system and peripheral lymph nodes have been described. For instance, the mucosal addressin cell adhesion molecule 1 (MAAdCAM-1) mediates the binding of gut-homing lymphocytes by interacting with the integrin α4β7 (Berlin et al., 1993).

Regulation of B cell adhesion and migration

Chemokines
The chemokines are a group of small secreted cytokines with chemotactic capacity (Baggiolini, 1997, 1998; Mantovani, 1999; Zlotnik and Yoshie, 2000). In addition, chemokines are involved in proliferation, apoptosis, and angiogenesis (D’Souza and Harden, 1996; Baggiolini, 1997, 1998; Berger et al., 1999). Chemokines are being secreted by a wide variety of cell types, including lymphocytes, dendritic

Table IV. Chemokine receptors expressed on B cells.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>B cell subpopulations</th>
<th>Ligand(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR2</td>
<td>naive and activated B cells</td>
<td>MCP-1, 2, 3, 4, 5</td>
</tr>
<tr>
<td>CCR5</td>
<td>immature, naive and activated B cells</td>
<td>RANTES, MIP-1α, -1β</td>
</tr>
<tr>
<td>CCR6</td>
<td>naive and activated B cells</td>
<td>MIP-3α</td>
</tr>
<tr>
<td>CCR7</td>
<td>immature, naive and activated B cells</td>
<td>MIP-3β, SLC</td>
</tr>
<tr>
<td>CXCR2</td>
<td>naive and activated B cells</td>
<td>IL-8, GROα, -β, -γ, NAP-2, ENA-78, GCP-2, LIX</td>
</tr>
<tr>
<td>CXCR4</td>
<td>immature, naive and activated B cells</td>
<td>SDF-1</td>
</tr>
<tr>
<td>CXCR5</td>
<td>naive and activated B cells</td>
<td>BCA-1</td>
</tr>
</tbody>
</table>

BCA, B cell attracting chemokine; CCR, CC chemokine receptor; CXCR, CXC chemokine receptor; ENA, epithelial neutrophil activating protein; GCP, granulocyte chemotactic protein; GRO, growth related oncogene; IL, interleukin; LIX, lipopolysaccharide induced CXC chemokine; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; NAP, neutrophil activating protein; RANTES, regulated upon activation, normally T cell expressed and secreted; SDF, stromal cell derived factor; SLC, secondary lymphoid tissue chemokine.
cells and stromal cells. To date, almost 50 chemokines have been characterized in man (Mantovani, 1999; Zlotnik and Yoshie, 2000). Chemokines are at least 20% homologues to each other and have been divided into 4 subgroups defined on the basis of a conserved cysteine motif near the N-terminus: C, CC, CXC, or CX3C. Chemokines can also be subdivided into homeostatic chemokines and inflammatory chemokines (Mantovani, 1999; Lindhout et al., 1999; Zlotnik and Yoshie, 2000). Generally, homeostatic chemokines regulate the homing of lymphocytes to lymphoid organs/microenvironments, while inflammatory chemokines are involved in the transient recruitment of leukocytes to sites of inflammation.

All chemokines bind and activate seven-transmembrane-spanning receptors expressed by many cells of the immune system, including all white blood cells, and dendritic cells (Baggiolini, 1997, 1998; Lindhout et al., 1999; Mantovani, 1999). Binding of a chemokine to its receptor induces G protein-dependent signal transduction, resulting in rearrangement of the cytoskeleton, and in integrin activation, ultimately leading to chemotaxis or other biological responses (Ben-Baruch et al., 1995; Bokoch, 1995; Kuang et al., 1996; Laudanna et al., 1996).

B cells can express several chemokine receptors including CCR7, CXCR4, and CXCR5 (see Table IV). CCR7 and its ligand macrophage inflammatory protein 3 β (MIP-3β) attract B and T cells, but not monocytes or granulocytes (Kim et al., 1998; Ngo et al., 1998). A second agonist for CCR7 is secondary lymphoid tissue chemokine (SLC). SLC is expressed by endothelial cells of HEVs and by stromal cells in the T cell areas of secondary lymphoid organs (Gunn et al., 1998b; Nagira et al., 1998). Both human and murine SLC are potent attractants for T cells and B cells (Gunn et al., 1998b; Nagira et al., 1997). Nagira et al. (1998) demonstrated that SLC is able to induce the transendothelial migration of large numbers of B cells and T cells in vitro. Remarkably, it was shown that rolling of T cells on HEVs of murine Peyer’s patches is dependent on SLC/CCR7, while rolling of B cells is not (Warnock et al., 2000). Mice deficient in CCR7 demonstrate a disturbed microarchitecture of all secondary lymphoid organs, probably as a result of impaired entry and retention of B cells, T cells, and dendritic cells (Förster et al., 1999). The deranged architecture of lymphoid organs in CCR7-deficient mice resulted in delayed humoral responses against DNP-KLH, and in absent contact sensitivity or delayed type hypersensitivity reactions.

CXCR4 and its ligand stromal cell-derived factor 1 (SDF-1) have also been described to be important for B cell functioning. SDF-1 was originally identified as a pre-B cell growth-stimulating factor (Nagasawa et al., 1994). Subsequently, it was demonstrated that it also acts as a chemoattractant for both lymphocytes and monocytes and that it attracts naive and memory B cells, but not germinal center B cells (Bleul et al., 1996, 1998; D’Apuzzo et al., 1997). Interestingly, by promoting CXCR4 internalization, activation of the B cell antigen receptor (BCR) inhibits
SDF-1-induced chemotaxis (Guinamard et al., 1999). In addition to mediating B cell migration, CXCR4 and/or SDF-1 are involved in B lymphopoiesis and myelopoiesis, angiogenesis, and cardiac and cerebellar development (Nagasawa et al., 1996; Nagasawa et al., 1996; Oberlin et al., 1996; Ma et al., 1998, 1999; Tachibana et al., 1998; Zou et al., 1998).

A chemokine receptor, highly expressed in murine B cells and Burkitt’s lymphomas, was cloned by Dobner et al. (1992). Disruption of the murine gene encoding this receptor, CXCR5, resulted in a severely impaired migration of B cells to follicles of the spleen and Peyer’s patches, and in the absence of inguinal lymph nodes (Förster et al., 1996). However, despite the disturbed architecture of their lymphoid organs, these mice demonstrate normal humoral immune responses and Ig affinity maturation upon immunization with T cell-dependent antigens (Förster et al., 1996; Voigt et al., 2000). Recently, a B cell-homing chemokine, called B cell attracting chemokine 1 (BCA-1) in humans and B lymphocyte chemotactant (BLC) in mice, was shown to be an agonist for CXCR5 (Gunn et al., 1998a; Legler et al., 1998). BLC is highly expressed in the follicles of secondary lymphoid organs and attracts B cells and to a much lesser degree T cells and macrophages (Gunn et al., 1998a). BCA-1 was shown to induce chemotaxis of B cells, but not activated T cells, monocytes, or neutrophils (Legler et al., 1998).

**Hepatocyte growth factor**

Hepatocyte growth factor (HGF), also known as scatter factor (SF), induces growth, motility, and morphogenesis of target epithelial and endothelial cells by binding to the receptor tyrosine kinase Met (Stoker et al., 1987; Nakamura et al., 1989; Bottaro et al., 1991; Montesano et al., 1991; Bladt et al., 1995; Schmidt et al., 1995; Uehara et al., 1995). Many studies suggest important roles for HGF in tumor growth, invasion, and metastasis (Weidner et al., 1990; Giordano et al., 1993; Rong et al., 1994). In addition, HGF was shown to be involved in hematopoiesis, adhesion of neutrophils, and in the migration of T cells (Kmieciak et al., 1992; Adams et al., 1994; Galimi et al., 1994; Nishino et al., 1995; Takai et al., 1997; Mine et al., 1998; Weimar et al., 1998). In Chapter 6 of this thesis an extensive overview of the structure, expression, and functions of HGF and Met is given. Data demonstrating a function for HGF/Met in B cell adhesion and differentiation will be presented and discussed in Chapter 3. In addition, the binding and presentation of HGF by heparan sulfate-modified CD44 to Met are shown and discussed in Chapters 4 and 5.
Heparan sulfate proteoglycans

The function of many cytokines, including the chemokines, can be modified by heparan sulfate proteoglycans (HSPGs). HSPGs form a subgroup of the family of proteoglycans, proteins that contain complex carbohydrate chains, called glycosaminoglycans (GAGs) (Kjellén and Lindahl, 1991). The GAG heparan sulfate (HS) is composed of a tetrasaccharide linkage unit covalently attached to a serine present in the core protein, followed by repeats of uronic acid (either D-glucuronic acid (GlcA) or L-iduronic acid (IdoA)) and D-glucosamine (GlcN) (Fig. 6). The GlcN units of HS can either be N-sulfated or N-acetylated (Fig. 6). Because of differences in the number and position of N-linked and O-linked sulfates, HS chains are often very heterogeneous (Fig. 6) (Kjellén and Lindahl, 1991; Lindahl et al., 1998; Bernfield et al., 1999). Additional HSPG variability is given by the large number of core proteins to which HS chains can be attached.

HSPGs are widespread throughout mammalian tissues both as cell surface molecules, e.g. the syndecans, glypicans, several CD44 isoforms, and as extracellular matrix (ECM) components, e.g. perlecan (Bernfield et al., 1992, 1999; David, 1993; Jackson et al., 1995; Iozzo, 1998). A number of cell biological and genetic studies have recently provided compelling evidence for an in vivo role of cell-surface HSPGs in growth control and morphogenesis in fruitflies, mice and humans (Selleck, 1998; Bernfield et al., 1999). For instance, proteoglycans in Drosophila bind the secreted glycoprotein wingless, and promote wingless-induced signal transduction (Reichsan et al., 1996; Binari et al., 1997). Moreover, mutation of a Drosophila glypican, or of enzymes required for the biosynthesis of HSPGs, lead to major developmental defects (Jackson et al., 1997; Lin et al., 1999). Deletion of the murine gene encoding the sulfation enzyme HS 2-sulfotransferase, resulted in developmental defects of the kidney, eye, and skeleton (Bullock et al., 1998). These data indicate the importance of correct HS synthesis and expression for murine development. In man, mutations in GPC3, the glypican-3 gene, cause the Simpson-Golabi-Benhmel syndrome, which is characterized by both pre- and postnatal overgrowth, a distinct facial appearance, and a diverse spectrum of other developmental defects (Pilia et al., 1996). This suggest that glypican-3 plays an important role in growth control during development. In addition to these functions in development, HSPGs have been implicated in cell adhesion and migration, angiogenesis, and in the regulation of blood coagulation (Jackson et al., 1991; Kjellén and Lindahl, 1991; Iozzo, 1998). During these processes, HSPGs bind ligands via their core protein, but more often via their HS chain(s). For instance, many cytokines, e.g. fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), and most, if not all, chemokines can bind to heparin, a highly sulfated GAG homologues to HS (Kjellén and Lindahl,
The binding of cytokines to HS chains has been shown to be highly specific and depends on the chemical composition and structure of the HS chains (Ishihara et al., 1993; Maccarana et al., 1993; Lyon et al., 1994; Guimond and Turnbull, 1999). Cytokine/HS interactions may serve a variety of functions ranging from immobilization and concentration, to distinct modulation of their biological function (Ruoslathi and Yamaguchi, 1991; Schlessinger et al., 1995). This functional importance is illustrated by fibroblast growth factor 2 (FGF-2), whose binding to its signal-transducing receptor and consequent biological effects is dependent on its interaction with cell-surface HSPGs (Rapraeger et al., 1991; Yayon et al., 1991). HS chains may bind and oligomerize FGFs, thereby promoting FGF receptor (FGFR) cross-linking and subsequent activation (Spivak-Kroizman et al., 1994; Schlessinger et al., 1995; DiGabriele et al., 1998). The structural model of a recently crystallized dimer of FGF-2 bound to a variant of FGFR1 suggested that heparin- (or HS-) induced dimerization represents the minimal structural unit required for activation of FGFRs (Plotnikov et al., 1999). Interestingly, this model also suggests that a heparin dodecasaccharide interacts with heparin-binding regions in both FGF-2 and FGFR1. An alternative function for HS is, that it induces conformational changes in FGFs, which might be neccessary to bind to FGFRs (Yayon et al., 1991). In addition, HS might protect
FGFs from degradation (Gospodarowicz and Chen, 1986; Saksela et al., 1988; Damon et al., 1989).

Most studies concerning the expression and function of cell-surface HSPGs have focussed on epithelial cells and fibroblasts, but these molecules presumably also play important roles in the immune system. For instance, a vast number of cytokines, involved in lymphoid tissue homeostasis or inflammation bind to HS/heparin. These cytokines, which include chemokines, as well as interleukins and hematopoietic growth factors, e.g. interleukin (IL) 3, IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF), and HGF, thus can be potentially immobilized by HSPGs (Gordon et al., 1987; Roberts et al., 1988; Webb et al., 1993; Lyon et al., 1994; Jackson, 1997; Gupta et al., 2000). Moreover, HSPGs expressed on the luminal surface of endothelial cells have been shown to bind chemokines produced at sites of inflammation (Tanaka et al., 1998), preventing their immediate dilution by the blood stream. Presentation of HSPG-bound chemokines, e.g. macrophage inflammatory protein (MIP) 1β and IL-8, to leukocytes plays a crucial role in activating the leukocyte integrins that mediate stable adhesion to and transmigration across the vessel wall (Rot, 1992; Tanaka et al., 1993a, 1993b, 1996). Chemokines and other heparin-binding cytokines do not exclusively act at the endothelial-blood interface. They also play key roles in the regulation of lymphocyte trafficking within lymphoid tissues and are involved in the control of lymphocyte growth, differentiation and survival (see also section Regulation of B cell adhesion and migration - Chemokines). This suggests that cell-surface HSPGs on cells of the immune system, such as lymphocytes and antigen-presenting cells, might also be involved in the regulation of cytokine responsiveness.

As yet, not much is known about the expression of HSPGs by B cells. In mice, precursor B cells present in the bone marrow, and plasma cells in interstitial matrices, have been shown to express the HSPG syndecan-1 (Sanderson et al., 1989). In vitro, expression of syndecan-1 was shown to mediate the binding of B cells to collagen (Sanderson et al., 1992) Also human plasma cells, in addition to myeloma cells, and Reed-Sternberg cells of classical Hodgkin's disease, express syndecan-1 (Ridley et al., 1993; Wijdenes et al., 1996; Carbone et al., 1997; Carbone et al., 1997). Ectopic expression of syndecan-1 in a human B cell line confers adhesion and spreading on thrombospondin and fibronectin (Lebakken and Rapraeger, 1996). However, this binding was shown to be mediated by the syndecan-1 core protein and not by HS chains. Also syndecan-4 has been shown to be expressed by murine B cells, with the interesting exception of Ig isotype switched B cells (Yamashita et al., 1999). So far the functional consequence of HS expressed by B cells is largely unknown, although HS has been reported to mediate IL-7-dependent B lymphopoiesis in vitro (Borghesi et al., 1999).
Aim of this study

The studies described in this thesis investigate the involvement of two types of proteins, *i.e.* the CD44 family of adhesion molecules, and the hepatocyte growth factor (HGF) receptor Met, in the regulation of B cell activation and adhesion.

The molecular structure of distinct members the CD44 family of adhesion molecules differs greatly as a result of alternative mRNA splicing and differential glycosylation. These molecules might bind to different ligands, or to the same ligand with distinctive affinity. In **Chapter 2**, the capacity of several alternatively spliced CD44 isoforms, to bind hyaluronic acid, a major CD44 ligand, was explored.

HGF and its receptor tyrosine kinase Met are involved in mitogenesis, motogenesis, and morphogenesis of epithelial and endothelial cells. Moreover, HGF plays a role in hematopoiesis, and in the adhesion and migration of T cells and neutrophils. In **Chapter 3**, we investigated the role of HGF/Met in B cell functioning. It describes the expression of Met on freshly isolated tonsillar B cells, and the secretion of HGF by cells present in lymphoid organs. In addition, we describe the effect of HGF on the adhesion of B cells to the extracellular matrix molecule fibronectin, and to vascular cell adhesion molecule 1 (VCAM-1), a molecule expressed by endothelial and follicular dendritic cells.

The action of many cytokines, including HGF, is regulated through their binding to heparan sulfate proteoglycans. CD44 isoforms containing a domain encoded by the alternatively expressed exon v3 can be modified with heparan sulfate (HS). Therefore, we investigated the influence of HS-modified CD44 (CD44-HS) on HGF functioning. Data concerning HGF-induced signal transduction in B cells expressing Met in combination with CD44-HS are described in **Chapters 4 and 5**. **Chapter 5** furthermore describes the delicately regulated expression of HSPGs, in particular CD44-HS, on tonsillar B cells.

**Chapter 6** comprises a review on the involvement of the HGF - Met pathway during development, tumorigenesis, and B cell differentiation.

References


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General introduction


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